Supplementary Material for

Rational Introduction of Electrostatic Interactions at Crystal Contacts to Enhance Protein Crystallization of an Ene Reductase

Brigitte Walla [1], Anna Maslakova [1], Daniel Bischoff [1], Robert Janowski [2], Dierk Niessing [2,3] and Dirk Weuster-Botz [1,*]

[1] Biochemical Engineering, Department of Energy and Process Engineering, TUM School of Engineering and Design, Technical University of Munich, Boltzmannstraße 15, 85748 Garching, Germany
[2] Molecular Targets and Therapeutics Center, Institute of Structural Biology, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany
[3] Institute of Pharmaceutical Biotechnology, Ulm University, James-Franck-Ring N27, 89081 Ulm, Germany
* Correspondence: dirk.weuster-botz@tum.de

Table S1. Forward (5'►3') and reverse (3'►5') oligonucleotide sequences for the site-directed mutagenesis of the selected *Nsp*ER1-L1,5 mutants *via* QuikChange PCR. Oligonucleotides were designed according to Zheng et al. [1] (mutation in bold).

***Nsp*ER1-L1,5 mutant**	**5' ► 3'**	**3' ► 5'**
Q171E	caatatcgc**gaa**gggggctgcaaac	gcccc**ttc**gcgatattgttcgac
Q204K	gacgaat**aaa**cgtacagacg	gtacg**ttt**attcgtcccatc
Q263K	gtagct**aaa**gcactgaaccgcttc	cagtgc**ttt**agctacgtaacc
A264K	gctcaa**aaa**ctgaaccgcttc	gttcag**ttt**ttgagctacgtaacc
D280K	gaagctata**aaa**gcagacattagac	gtctgc**ttt**atagcttcaaag
V344E	ctagag**gaa**aacgcaccactc	gtgcgtt**ttc**ctctaggcgttc
D350K	cactcaat**aaa**gcagaccccacaac	gtctgc**ttt**attgagtggtgcgttc
D352K	caatcaggca**aaa**cccacaacct	gtggg**ttt**tgcctgattgagtggtg
T354K	gacccca**a**aacctttatggtgg	aaaggttttggggtctgcctgattg

Table S2. Data collection and refinement statistics of X-ray diffraction experiments of crystals from the *Nsp*ER1-L1,5 wild type and mutants Q204K, Q350K, D352K, T354K (values in parentheses are for highest resolution shell).

Variant	**Wild type**	**Q204K**	**Q350K**	**D352K**	**T354K**
Data collection					
PDB ID	9QGB	9QGC	9QGD	9QGE	9QGF
Beamline	SLS Beamline X06SA	PETRA III, DESY BEAMLINE P11	PETRA III, DESY BEAMLINE P11	PETRA III, DESY BEAMLINE P11	PETRA III, DESY BEAMLINE P11
Wavelength, Å	1.00	1.03	1.03	1.03	1.03
Space group	P 1 21 1	P 21 21 21	P 21 21 21	P 1 21 1	P 1 21 1
Cell constants a, b, c, α, β, γ	55.2Å 67.8Å 91.5Å 90.0° 93.5° 90.0°	55.4Å 68.6Å 90.0Å 90.0° 90.0° 90.0°	55.3Å 68.4Å 89.9Å 90.0° 90.0° 90.0°	55.4Å 68.2Å 91.63Å 90.0° 94.1° 90.0°	55.5Å 68.1Å 91.71Å 90.0° 93.7° 90.0°
Chains per asymmetric unit	2	1	1	2	2
Resolution, Å	48.8-1.27 (1.34-1.27)	48.8-1.24 (1.31-1.24)	48.8-1.24 (1.31-1.24)	48.8-1.43 (1.52-1.43)	48.8-1.44 (1.53-1.44)
CC (1/2)	1.00 (0.65)	1.00 (0.52)	1.00 (0.73)	1.00 (0.57)	1.00 (0.53)
Variant	**Wild type**	**Q204K**	**Q350K**	**D352K**	**T354K**
Completeness, %	97.5 (90.9)	99.5 (97.0)	98.5 (91.3)	98.3 (96.8)	98.8 (98.3)

Redundancy	6.75 (5.60)	9.31 (6.66)	12.57 (9.88)	5.12 (4.98)	6.84 (6.54)
Refinement					
Resolution, Å	1.27	1.24	1.24	1.43	1.44
R_{work} / R_{free}	0.13 / 0.16	0.13 / 0.16	0.12 / 0.15	0.15 / 0.20	0.14 / 0.19
No. atoms	7065	3482	3575	6661	6781
Protein	5743	2947	2987	5706	5749
Water	1160	472	532	882	900
Other	162	63	56	73	132
B-factors					
Overall	16.0	18.0	20.0	17.0	17.0
Protein main chain	11.96	14.33	15.28	14.14	13.94
Protein side chain	14.09	16.61	17.73	16.02	15.82
R.m.s. deviations					
Bond lengths, Å	0.01	0.01	0.01	0.01	0.01
Bond angles, °	1.91	1.92	2.10	1.80	1.83
Ramachandran plot					
Most favored, %	92.72	93.00	93.56	92.86	92.84
Additional allowed, %	6.72	5.88	5.60	6.16	6.60

Table S3. List of the root-mean-square deviation (RMSD) of Cα atoms (Å) and chain alignments for the *Nsp*ER1-L1,5 wild type and mutants Q204K, Q350K, D352K, and T354K.

RMSD (Å)	Wild type (A,B)	Q204K (D)	Q350K (D)	D352K (A,B)	T354K (A,B)
Crystal environment alignment					
wild type (A)	0, 2.84	9.17	9.15	2.92, 0.34	2.84, 0.27
wild type (B)	2.84, 0	10.2	10.19	0.33, 2.92	0.27, 2.84
Monomer alignment					
wild type (A)	0.00, 0.11	0.10	0.11	0.11, 0.07	0.11, 0.05

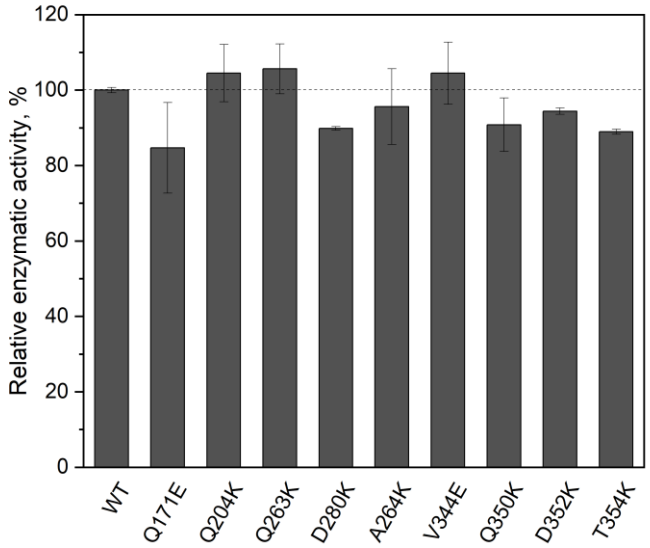


Figure S1. Maximum enzymatic activity of *Nsp*ER1-L1,5 mutants Q171E, Q204K, Q263K, A264K, D280K, V344E, D350K, D352K, and T354K relative to the *Nsp*ER1-L1,5 wild type (WT, set to 100%). IMAC-purified protein solutions of *Nsp*ER1-L1,5 variants were adjusted to 60 mg L^{-1}. Enzymatic activity was measured spectrophotometrically for 10 min at 340 nm (30 °C, every 6 sec) with addition of 180 µL buffer (120 mM NaH_2PO_4/ Na_2HPO_4, pH 7.0) containing 0.2 mM NADH and 10 mM maleimide to a final volume of 200 µL (n = 5).

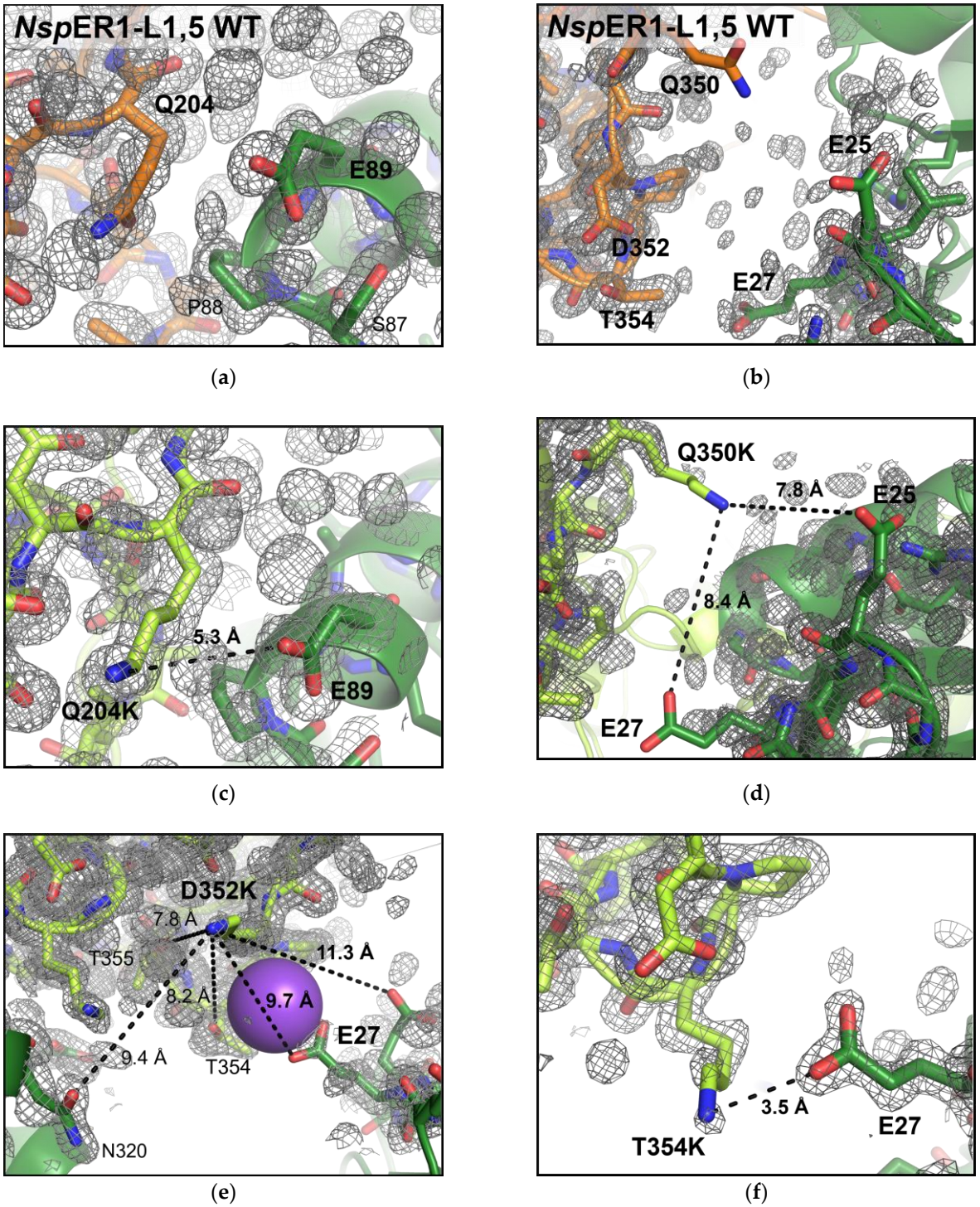


Figure S2. Electron density map (grey) of *Nsp*ER1-L1,5 crystal contact positions (**a**) Q204 and (**b**) 350-354, as well as of the respective mutants (**c**) Q204K, (**d**) Q350K, (**e**) D352K, and (**f**) T354K with well-defined electron density of the lysine and glutamic acid side chain for Q204K and T354K. Small density spheres represent water molecules. The map represents the structure factor amplitude difference $2F_o - F_c$ with a contour level of 1.0 σ (calculated with REFMAC [2]).

Reference

1. Zheng, L., Baumann, U., and Reymond, J. L. "An efficient one-step site-directed and site-saturation mutagenesis protocol." *Nucleic Acids Res* 32, no. 14 (2004): e115. 10.1093/nar/gnh110

2. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. "Refinement of macromolecular structures by the maximum-likelihood method." *Acta Crystallographica Section D* 53, no. 3 (1997): 240-55. doi:10.1107/S0907444996012255